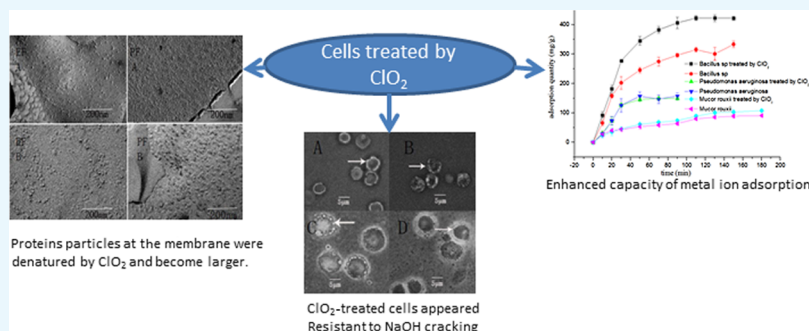


Application of Chlorine Dioxide in Cell Surface Modification to Enhance Its Mechanical Stability and Metal Ion Adsorption

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ABSTRACT: There has been a trend toward the use of microorganisms as the biomaterial for removing dyes and metals from wastewater. However, native microorganism cells have low mechanical stability, which limit their further application in industries. In this study, chlorine dioxide (ClO_2), a high-efficiency, low-toxicity, and environmentally benign disinfectant, was used for microorganism surface modification to enhance the mechanical stability and metal ion adsorption of the cell. ClO_2 can either modify cell walls to improve their metal adsorption capacity or modify cell membranes to improve their mechanical stability. Fourier-transform infrared spectroscopy analysis indicated that several cell surface groups were involved in the cell wall modification of *Bacillus* sp. Microscopic observation indicated that ClO_2 treatment could deter cell membranes from forming vesicles in sodium hydroxide (NaOH) aqueous solution, and freeze-etching showed that ClO_2 treatment could alter the erythrocyte membrane proteins which might also contribute to improving the cell stability. The experimental results on *Bacillus* sp., *Pseudomonas aeruginosa*, and *Mucor rouxii* show that ClO_2 treatment may increase, or at least not reduce, the ability of microbial cells to adsorb heavy metals, but it can significantly improve the resistance of these cells to NaOH cleavage. It seems ClO_2 is a promising auxiliary for biosorption of heavy-metal ions.

1. INTRODUCTION

Both dyes and heavy metals are common contaminants in industrial wastewaters and many of them are known to be toxic and carcinogenic.^{1,2} Hence, effective and thorough methods for remediating dye- and metal-contaminated water are urgently needed. Of the various techniques employed for the treatment of dye- and metal-bearing industrial effluents, biosorption has become a favorable biotic method of choice because of its environmentally benign approach, good performance, low cost, and large available biomass quantities.^{3–5}

Many raw microorganisms have been reported to be capable of accumulating dyes and/or metals.^{2,3} However, further research has revealed that cell biomass can be pretreated to improve cell adsorption capacity by surface group modification.⁴ A number of methods have been employed for cell wall modification, among which NaOH treatment has been widely used because of its positive effect on biosorption.^{6–9} NaOH has also been used for desorption or pretreatment to regenerate the biosorbents, thus playing an important role in their further industrial application.¹⁰

However, NaOH has an intense cell lysis effect on the cells of many organisms. Yan observed that NaOH pretreatment

resulted in a dramatic increase in metal adsorption capacity of *Mucor rouxii* and a significant reduction in biomass compared to an autoclaved biomass control.⁹ Other reagent treatments such as NaCO_3 , CaCl_2 , HCl , and H_3PO_4 have also demonstrated a significant cracking effect on *M. rouxii* cells.¹¹ Dow and Rubery found that cell walls of *M. rouxii* could be ruptured using NaOH.¹² Fourest and Volesky reported that up to a 39% biomass loss could be achieved by pretreating *Sargassum fluitans* with NaOH.¹³ The mass loss of biomass during pretreatment may lead to some confusion during the quantitative assessment of the bioadsorption performance.¹⁴ In addition, the poor mechanical stability of the native microorganisms may have limited their further application under real conditions.^{15,16} Hence, it is important to improve the stability of microorganisms before their further application in industries. As far as the authors know, immobilization was the only method to improve the mechanical stability of the microorganisms.¹⁷

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In the current study, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus* sp., *M. rouxii*, and erythrocytes, which represent Gram-negative bacteria, Gram-positive bacteria, fungi, and animal cells, respectively, were treated by ClO_2 . We demonstrate that ClO_2 , a high-efficiency, low-toxicity, and environmentally benign disinfectant, could be used for *Bacillus* sp. B26 and *M. rouxii* modification to improve their metal adsorption capacity. More importantly, we were very lucky to find out that ClO_2 can modify different kinds of cell membranes to enhance their stability in an aqueous NaOH solution. Therefore, ClO_2 is a promising regeneration aid for those who use NaOH to treat biosorbent materials for regeneration but are trapped by the degradation of the cells by NaOH.

2. MATERIALS AND METHODS

2.1. Culture Media and Harvesting Techniques.

Bacillus sp. (Gram-positive bacteria), *E. coli* (Gram-negative bacteria), *P. aeruginosa* (Gram-negative bacteria), and *M. rouxii* (fungus) were purchased from Guangdong Microbial Culture Collection Center (GMCCC, China). The growth medium for *Bacillus* sp. was prepared as described by Xu,¹⁸ which contained maltose 10.0 g/L, casein peptone 10.0 g/L, beef extract 5.0 g/L, and NaCl 2.0 g/L, and was maintained at pH 7.2 and 32 °C with an agitation speed of 200 rpm in a rotating shaker.¹⁷ The growth medium for *M. rouxii* contained 50.0 g/L glucose, 20.0 g/L casein peptone, 2.0 g/L yeast extract, 4.0 g/L KH_2PO_4 , and 2.0 g/L MgSO_4 , and was maintained at pH 4.5 and 28 °C with an agitation speed of 300 rpm in a rotating shaker. The growth medium for *E. coli* and *P. aeruginosa* contained 10.0 g/L casein peptone, 5.0 g/L yeast extract, and 10.0 g/L NaCl, and was incubated at pH 7.0 and 37 °C with an agitation speed of 120 rpm in a rotating shaker. Cells were harvested at a suitable time by centrifugation at 6000g for 10 min at 4 °C and washed three times with double-distilled water (ddH_2O), and then resuspended with ddH_2O at a concentration of 10^9 CFU/mL for subsequent experiments. Erythrocytes were harvested from rabbit flesh blood by adding 5 mL of flesh blood to 0.4 mL EDTA- Na_2 (15 g/L). The fresh erythrocytes were washed three times with physiological saline and adjusted to 2% concentration (v/v) for subsequent use.

2.2. Metal Ion Adsorption Experiments on ClO_2 -Treated Cells. For the treatment of cells with ClO_2 , the bacteria suspension of 1 mL was transferred to a centrifuge tube. Then, the tube was centrifuged to pellet the cells and discard the supernatant completely. The cells were resuspended with 1 mL ClO_2 solution (150 mg/L) and incubated for 10 min at room temperature. After that, the cells were washed with ddH_2O to remove the residual ClO_2 .

Uranium is one of the most seriously hazardous heavy metals because of its high toxicity and some radioactivity. Considerable amounts of uranium have found their way into the environment through the activities associated with the nuclear industry. Wet biomasses (0.01 g or an equal cell-immobilized matrix) were added to 30 mL solutions containing UO_2^{2+} (300 mg/L, for *Bacillus*), Cu^{2+} (300 mg/L, for *P. aeruginosa*), and Fe^{3+} (300 mg/L, for *M. rouxii*). The suspensions were incubated at 25 °C and pH 6.0 in a shaker at 180 rpm and sampled periodically. The collected samples were centrifuged at 6000g for 10 min and the supernatant was then filtered through a 0.45 μm cellulose membrane. The residual uranium on the filter was measured with a micro-uranium analyzer with a detection range of 3.0×10^{-11} to 2.0×10^{-5} g/mL and a relative standard deviation of less than or equal to

8%. Atomic adsorption spectrometry was used for detection of Cu^{2+} and Fe^{3+} .

2.3. Fourier Transform Infrared Analysis of *Bacillus* sp. B26 Biomass. Suspensions (2 mL in each centrifuge tube) of *Bacillus* sp. B26 were centrifuged at 4 °C, 8000g for 5 min, the pellets were washed three times and resuspended in lyoprotective solution, stored at −70 °C for 2 h, and then lyophilized for 12 h. Lyophilized biomass samples were characterized using Fourier transform infrared (FTIR) analysis to better understand the modification effect of ClO_2 on *Bacillus* sp. cells. Infrared spectra of the pure/uranium-bearing and ClO_2 -treated lyophilized *Bacillus* sp. biomass samples were recorded in the range of 4000–400 cm^{-1} on a Nicolet FTIR spectrophotometer using KBr pellets.

2.4. NaOH Cell Lysis Experiments. The cell sediments of *Bacillus* sp., *E. coli*, *M. rouxii*, and erythrocyte were subjected to NaOH additions of different final concentrations ranging from 0 to 100 mmol/L and shaken in suspension for 10 min. The NaOH-treated cells were harvested by centrifugation and resuspended in distilled water (physiological saline for erythrocytes), and then subjected to cell concentration determination by detecting OD_{600} values (by UV–vis spectrophotometry at 600 nm, Beijing Puxi TU-1810) for *Bacillus* sp. and *E. coli*, and using a hematocyte counter for *M. rouxii* and erythrocyte.

2.5. Resistance of ClO_2 -Treated Cells to NaOH. The cell sediments of *E. coli*, *M. rouxii*, and erythrocytes were subjected to ClO_2 additions of different final concentrations ranging from 0 to 150 mg/L and shaken in suspension for 10 min. The suspensions were centrifuged to remove residual ClO_2 , and the obtained cell sediments were treated with NaOH (100 mmol/L) as described in Section 2.4.

An addition experiment was carried out to observe NaOH cracking effects on ClO_2 -treated erythrocytes: erythrocytes were treated by ClO_2 , ClO_2 and NaOH, and NaOH, respectively; then, the suspensions were centrifuged to observe the volumes of sediments. The control erythrocytes were suspended only in normal saline, ClO_2 and NaOH concentration herein is 130 mg/L and 100 mmol/L respectively (normal saline as solvent).

2.6. Microscopic Observation and Freeze-Etching of Erythrocytes. A drop of erythrocyte suspension was dropped on the glass slide, and a cover slip was added. The prepared sample was observed under a phase contrast microscope. For freeze-etch experiments, the various fixed erythrocyte samples were frozen in liquid Freon 22 (−150 °C), transferred to liquid nitrogen, fractured, and then etched at −100 °C for 4 min in a high-vacuum evaporator (HUS-SGB) device, which was described by Wise,¹⁹ and observed under an H-600 transmission electron microscope.

3. RESULTS AND DISCUSSION

3.1. Effect of ClO_2 Treatment on Metal Adsorption by Different Microorganisms. The results of the time course experiments that examined metal adsorption with and without pretreatment with ClO_2 are shown in Figure 1. *Bacillus* sp. showed considerable adsorption of UO_2^{2+} within the first 30 min for both pretreated and untreated cells. Maximum adsorption is reached at about 120 min with an adsorption quantity of about 316.0 mg/L for the control group and 422.0 g/L for the ClO_2 -treated group. This demonstrated a 33.5% higher UO_2^{2+} ion adsorption capacity for the ClO_2 -modified *Bacillus* sp. cells relative to the control group. The adsorption

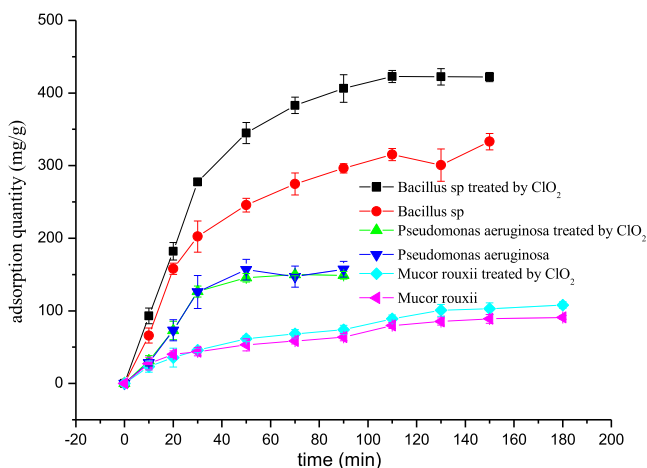


Figure 1. Effect of ClO_2 treatment on metal adsorption for different microorganisms. *Bacillus* sp.: UO_2^{2+} ; *P. aeruginosa*: Cu^{2+} ; *M. rouxii*: Fe^{3+} (error bars represent standard deviation).

capacity of *M. rouxii* for Fe^{3+} was 18.7% higher than that of the control at 180 min, whereas no difference in adsorption of Cu^{2+} by *P. aeruginosa* was observed between the experimental and control setups. Therefore, *Bacillus* sp. was chosen as the type of strain to study the cell wall modification mechanism by FTIR analysis.

As the biosorption process consists primarily of cell surface sequestration, the modification of cell walls can greatly alter the metal ion-binding mechanism.²⁰ The modification results may show enhancement or reduction in metal bioadsorption, depending on the fungal strains and treatment procedures used.⁹ The most widely used modification methods are heating, inorganic treatment (e.g. acid or caustic soda), and organic treatment (e.g. methanol or formaldehyde).^{7,21–23} However, chlorine dioxide, a promising disinfectant with high biocidal effects and low toxicity, has not previously been investigated to our knowledge. Compared to other pretreatment reagents, chlorine dioxide is more economical and more environmentally friendly.

3.2. FTIR Analysis of *Bacillus* sp. Experimental Samples. To examine the effect on binding of metals in the presence and absence of ClO_2 , Fourier transformed infrared spectra of *Bacillus* sp. B26 biomass of three treatment conditions, no treatment cells, UO_2^{2+} -loaded cells, and ClO_2 -treated cells, were examined. As shown in Figure 2A, the spectrum of the native biomass showed a characteristic peak in the 3749 cm^{-1} region because of the stretching of the O–H bonds in the carboxylic acid groups present in the biomass.²⁴ In the UO_2^{2+} -treated spectrum (Figure 2B), this peak was shifted to 3751 cm^{-1} , which could be attributed to carboxylic acid binding to UO_2^{2+} . In the ClO_2 -treated spectrum (Figure 2C), the O–H stretching peak was shifted to 3743 cm^{-1} , indicating its contribution to affecting the UO_2^{2+} adsorption by affecting the O–H bond of carboxylic acids. A peak in the $3280\text{--}3320\text{ cm}^{-1}$ (3307 , 3311 , and 3294 cm^{-1} for the no treatment, the UO_2^{2+} -loaded, and the ClO_2 -treated cells, respectively) was observed because of the stretching of the N–H bond of amino groups. This N–H stretching peak lies in a region occupied by a broad and strong band ($3200\text{--}3500\text{ cm}^{-1}$) that is due to the presence of $\gamma\text{O–H}$ of the hydroxyl groups.²⁵ For both the UO_2^{2+} -treated and ClO_2 -treated samples, changes in the peak position and shape in these spectra indicated that ClO_2 treatment may have influenced the

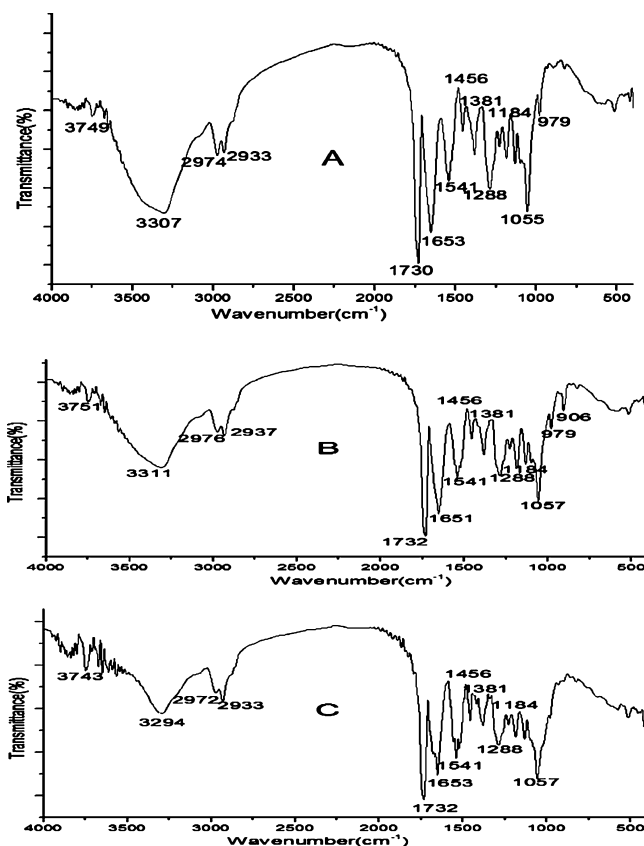


Figure 2. FTIR spectra of *Bacillus* sp. B26 biomass. (A) No treatment cells; (B) UO_2^{2+} -loaded cells; (C) ClO_2 -treated cells.

binding of uranium with amino and hydroxyl groups. In the control spectrum, the complex adsorption at $2900\text{--}3000\text{ cm}^{-1}$ is ascribed to the asymmetric stretching of the $\gamma\text{C–H}$ bonds in $-\text{CH}_2$ groups combined with that of the CH_3 groups.²⁶ Both peak position and shape changed in these regions for the UO_2^{2+} -treated and ClO_2 -treated samples. In the control spectrum, the peak at 2974 cm^{-1} is a little more intense than the peak at 2933 cm^{-1} , whereas the 2933 cm^{-1} peak is more intense than the 2974 cm^{-1} peak for the UO_2^{2+} -treated and ClO_2 -treated samples. There is a small shift in peak position from 1730 to 1732 cm^{-1} for the UO_2^{2+} -treated and ClO_2 -treated samples relative to the control.

All three spectra revealed the presence of protein-related bands. The $\gamma\text{C=O}$ of amide I ($1700\text{--}1600\text{ cm}^{-1}$) and $\delta\text{NH}/\gamma\text{C=O}$ combination of amide II ($1600\text{--}1500\text{ cm}^{-1}$) bonds were prominent at about 1653 and 1541 cm^{-1} , respectively.²⁷ Compared to the metal-free control cells and ClO_2 -treated cells, the spectrum for the UO_2^{2+} -treated samples showed a shift in the position of the 1653 cm^{-1} peak to 1651 cm^{-1} , indicating that ClO_2 treatment had no influence on sorption of U to the $\gamma\text{C=O}$ bond within amide I. The strong adsorption peaks between 1000 and 1100 cm^{-1} also indicated the presence of carboxyl groups in the bacterial polysaccharide structure. After metal binding by the biomass, minor changes in the peak position (1055 cm^{-1} in the control compared to 1057 cm^{-1} in the UO_2^{2+} -treated and ClO_2 -treated spectra) in this region strongly suggest the involvement of carboxyl groups in U sorption and ClO_2 treatment.²⁷ In the UO_2^{2+} -treated sample, the distinct peak at 906 cm^{-1} could be assigned to the asymmetric stretching vibration of $\nu_3\text{UO}_2^{2+}$. All peaks in the

1540–1000 cm^{-1} region were consistent in position across the three sample spectra.

3.3. Lysis Effects of the NaOH Solution on Erythrocytes, *E. coli*, *M. rouxii*, and *Bacillus* sp. Cells. In order to understand the preliminary lysis effects of NaOH on cells, four kinds of cells, erythrocytes, *E. coli*, *M. rouxii*, and *Bacillus* sp., were treated with NaOH at different concentrations. As shown in Figure 3, the erythrocytes began to lyse when the

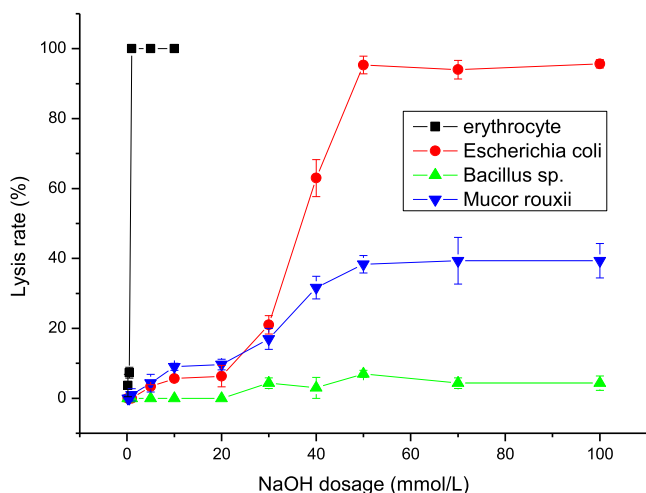


Figure 3. Lysis effect of NaOH on different cells (error bars represent standard deviation).

NaOH concentration reached 2 mmol/L, and cracked completely when NaOH concentration reached 5 mmol/L. A much higher concentration of NaOH was required to crack *E. coli*, with only 23% lysis occurring at about 30 mmol/L. The lysis rate for *E. coli* reached a maximum at about 95% when the NaOH concentration reached 50 mmol/L, an order of magnitude higher than that observed for the erythrocytes. The maximum lysis rate for *M. rouxii* was about 42% when the NaOH concentration was above 50 mmol/L, and the NaOH (ranging from 0 to 100 mmol/L) appeared to have no significance lysis effect on *Bacillus* sp. cells.

These four cell types were chosen for the lysis experiments because they represent four different cell surface structures. *Bacillus* sp. are Gram-positive bacteria comprising a thick peptidoglycan layer and *E. coli* are Gram-negative bacteria comprising a much thinner peptidoglycan layer.⁴ *M. rouxii* are fungal, and thus different from bacteria, and comprise primarily chitin, glucan, mannan, and protein layers, depending upon the species.²⁸ Erythrocytes are membrane-naked because of the absence of a proper cell wall and are more vulnerable to the external environment. The results showed that the susceptibility of cells to NaOH lysis decreases in the following order: erythrocyte > *E. coli* > *M. rouxii* > *Bacillus* sp. Erythrocytes, *E. coli*, and *M. rouxii* were chosen for the following study.

3.4. Tolerance of ClO_2 -Treated Cells to Cracking by NaOH. As shown in Figure 4, the ClO_2 treatments had no significant influence on the cell concentration of any of the three cell types tested. In all cases, the ClO_2 treatment had a positive dosage-dependent effect on the tolerance of cells to cracking by NaOH. In the erythrocytes group (Figure 4C), the cells showed dramatic tolerance to NaOH when the ClO_2 concentration reached 30 mg/L, whereas *E. coli* (Figure 4A) and *M. rouxii* (Figure 4B) only showed initial effects of ClO_2 dosages at 60 and 90 mg/L, respectively. When treated with

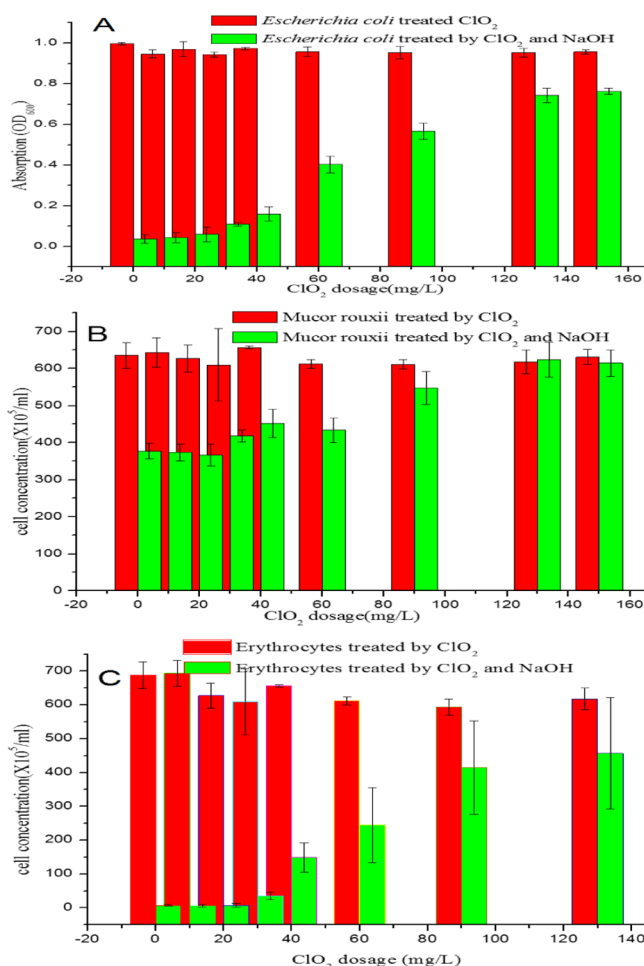


Figure 4. Resistance effect of ClO_2 -treated cells to NaOH lysis (NaOH was 100 mmol/L). (A) *E. coli*; (B) *M. rouxii*; (C) erythrocytes (error bars represent standard deviation).

ClO_2 at 130 mg/L, about 80% of the *E. coli* and erythrocyte cells were resistant to cracking by NaOH, whereas *M. rouxii* cells were 100% resistant.

Figure 5 shows images of the erythrocyte cell sediment samples after treatment with different reagents and centrifugation.

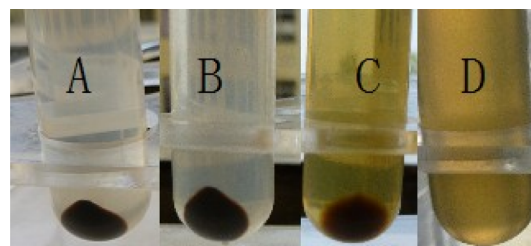


Figure 5. Tolerance of ClO_2 -treated cells to NaOH. Centrifuged erythrocyte sediments after (A) no treatment; (B) ClO_2 treatment (130 mg/L); (C) ClO_2 treatment (130 mg/L) and NaOH treatment (100 mmol/L); (D) NaOH treatment (100 mmol/L).

gation. There was no significant difference between the control group (Figure 5A) and ClO_2 -treated group (Figure 5B) with both displaying a white-transparent supernatant. Figure 5D had no sediment, indicating that all the cells were cracked by NaOH. The weak color in the Figure 5C supernatant may have

resulted from partial cracking of cells or leakage of hemoglobin after treatment by ClO_2 and NaOH.

It is of great interest that the ClO_2 -treated cells showed resistance to cracking by NaOH without cell species specificity. Because all three cell types have a biofilm composed of a phospholipid bilayer in common, it seems likely that the resistance results from membrane alteration. Jacobsohn reported that high or low pH may decrease the stability of biomembranes by exposing their hydrophobic domain to the aqueous phase, leading to changes in the membrane from a bilayer structure to micro-capsule structure.²⁹ As erythrocytes are immune to cell wall interference and are more visible under the microscope, samples of erythrocyte were observed and analyzed by a phase contrast microscope, flow cytometry, and transmission electron microscopy (TEM) to explore the action mechanism of ClO_2 on cell membranes.

3.5. Action Mechanism Analysis. Figure 6 shows the phase contrast microscope morphologies of erythrocytes

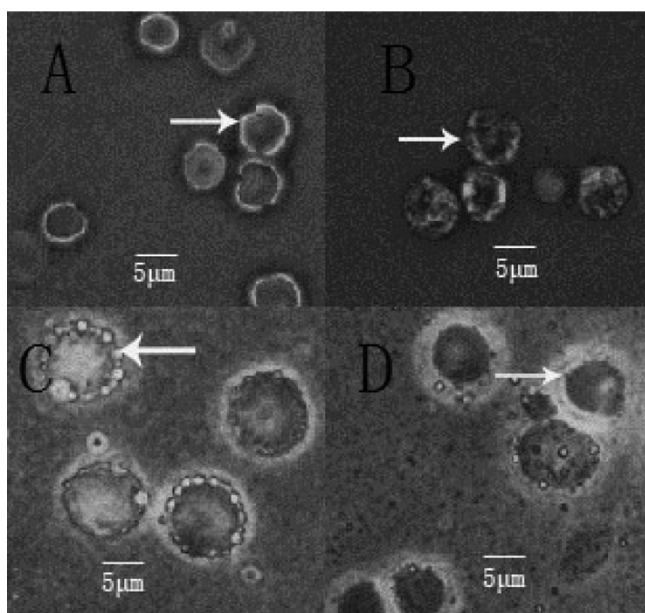


Figure 6. Morphology of erythrocytes ($\times 1000$). (A) No treatment; (B) ClO_2 treatment (130 mg/L); (C) ClO_2 treatment (30 mg/L) and NaOH treatment (100 mmol/L); (D) ClO_2 treatment (130 mg/L) and NaOH treatment (100 mmol/L).

treated with different reagents. The surface gloss of the erythrocytes changed greatly after ClO_2 treatment (Figure 6A,B). Many cracking cells were observed after treatment with 30 mg/L ClO_2 followed by treatment with 100 mmol/L NaOH (Figure 6C), which also testify an aspect of morphology changes in that high pH values may lead to changes in the membrane structure from a bilayer structure to a micro-capsule structure.³⁰ As shown in Figure 6D, the extent of cell vacuolation decreased notably after treatment with a high concentration of ClO_2 (130 mg/L).

Flow cytometry was used to investigate and detect erythrocytes, and two detectors were aimed at the point where the stream passes through the light beam: a forward scatter (FS) detector in line with the light beam and several side scatter (SS) detectors perpendicular to the light beam. By analyzing fluctuations in brightness at each detector, it is possible to derive information regarding the physical and chemical structure of each individual particle. The FS

brightness generally has a positive relationship with cell volume and the SS brightness depends on the complexity of the particle, such as the membrane roughness. As shown in Figure 7, the average SS brightness of the erythrocytes

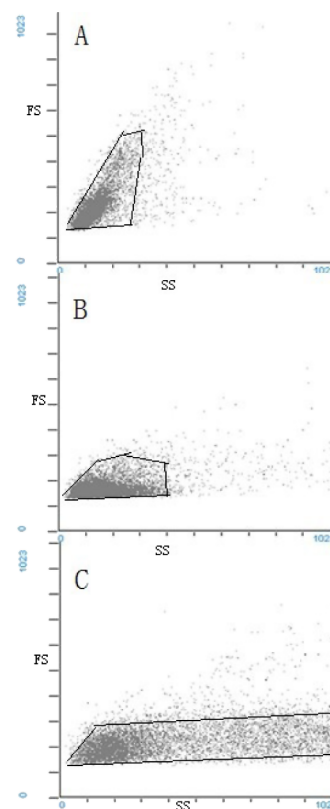


Figure 7. FS–SS dot plot of erythrocytes. (A) No treatment; (B) ClO_2 treatment (30 mg/L); (C) ClO_2 treatment (130 mg/L).

increased significantly with the increasing ClO_2 dosage, which, to some extent, indicates a change in membrane roughness. The average FS brightness of the ClO_2 -treated erythrocytes decreased relative to that of the control, indicating membrane constriction after ClO_2 treatment.

Figure 8 shows examples of freeze-etched profiles of normal erythrocytes and ClO_2 -treated erythrocytes. The extracellular fracture face (EF) and protoplasmic fracture face (PF) were distinguished according to their particle intensity: sparse ones are EF faces and intense ones are PF faces.³¹ The protein particles in normal erythrocytes (Figure 8A) were small (about 10 nm in diameter), whereas the ClO_2 -treated protein particles (Figure 8B) were 2–3 times larger, possible due to denaturation of proteins in the particles by ClO_2 treatment. Some of the ClO_2 -treated nearby particles seem to clump together. The above results suggested that ClO_2 treatment can greatly alter the erythrocyte membrane proteins, which may in some way contribute to its resistance to NaOH cracking. These findings also indicated that the cause for resistance of microorganisms to NaOH lysis may be primarily related to cell membrane alterations.

With regard to the molecular mechanisms, not much is known about organic by-products formed after ClO_2 treatment. As ClO_2 oxidizes proteins and lipids, membrane resistance to cracking could be enhanced by the ways in which the structures of membrane proteins and lipids are altered.³² Gierer reported that in the ClO_2 oxidation process,

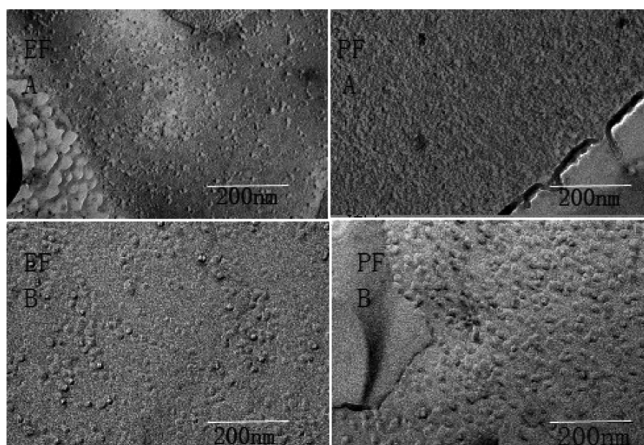


Figure 8. Freeze-etching micrographs of erythrocytes. (A) No treatment, (B) ClO_2 treatment (130 mg/L) EF: extracellular fracture face; PF: protoplasmic fracture face.

radical cation intermediates form immediately after the elimination reaction and the charge-transfer complex, during which chlorous acid and hypochlorous acid are produced.^{33,34} It is well known that cross linking of proteins and lipids happens during lipid peroxidation of cells, which is mediated by free radicals.³⁵ On the basis of previously published results, two proposed reaction pathways are presented in Scheme 1. In both proposed pathways, the cross-linking reactions are initiated by a hydrogen elimination reaction. In pathway A, the alpha carbon of the amino acid residue connects with the carbon of the amide bond. In pathway B, the peroxidized fatty acid cross links to another free radical containing a fatty acid through C–O bonds. Therefore, cross linking does not necessarily result in adsorption loss relative to other functional groups like $-\text{NH}_3$ or $-\text{COOH}$, but rather leads to an enhancement of the cell membrane adsorption capacity.

4. CONCLUSIONS

This study presents the first report of ClO_2 in its use in microorganism surface modification. FTIR analysis indicated that several cell wall groups were involved in cell wall modification of *Bacillus* sp. When treated with NaOH,

NaOH had an intense lysis effect on erythrocytes and *E. coli*, a notable lysis effect on *M. rouxii*, but no lysis effect on *Bacillus* sp. In all cases, the ClO_2 treatment had a positive dosage-dependent effect of prevented lysis, increasing the resistance of cells to lysis with NaOH. Microscopic observation indicated that ClO_2 treatment could deter membranes from forming vesicles in NaOH solution. Flow cytometry analysis indicated that constriction of erythrocyte membranes occurs after ClO_2 treatment. Freeze-etching showed that ClO_2 treatment could greatly alter the erythrocyte membrane proteins which might help improve the cell resistance to lysis by NaOH. These findings demonstrate that ClO_2 has great potential to be applied in microorganism modification to improve their dyes and metal adsorption capacity as well as enhance their stability.

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Notes

The authors declare no competing financial interest.

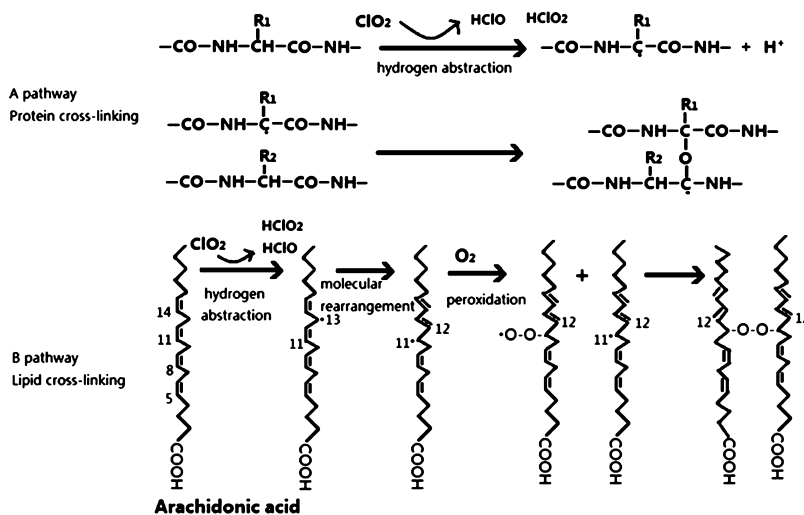
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Scheme 1. Two Proposed Molecular Reaction Pathways



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